Enhancement of Soluble CD23 Serum Levels and Cell-surface CD23-Expression in Subjects at Increased Risk of Type 1 Diabetes Mellitus and in Diabetic Patients

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The low affinity receptor for IgE, CD23, is expressed on lymphocytes among other cell types. The purpose of the present study was to assess serum sCD23 levels and CD23 expression on peripheral blood mononuclear cells (PBMC) in people at increased risk of developing Type 1 diabetes mellitus and in diabetic patients. Serum sCD23 levels were significantly higher in first-degree relatives of Type 1 patients (median: 3.2 U ml^{-1}) ($p < 0.001$) and in newly diagnosed (median: 3.3 U ml^{-1}) ($p < 0.001$) and long-standing (median: 2.5 U ml^{-1}) ($p = 0.01$) Type 1 diabetic patients than in controls (median: 1.2 U ml^{-1}). Newly diagnosed patients showed higher levels than those with long-standing disease ($p = 0.026$). Moreover the percentage of B cells expressing CD23 were significantly higher in first-degree relatives (median: 48.6 %) ($p < 0.001$) and in newly diagnosed (median: 58 %) ($p < 0.001$) and long-standing (median: 44.8 %) ($p = 0.03$) Type 1 diabetic patients than in controls (median: 28.5 %). The increased sCD23 levels and the increased number of cells expressing CD23 observed in subjects at increased risk of Type 1 diabetes and diabetic patients may be indicators of Th2 activity in Type 1 diabetes. © **1998 John Wiley & Sons, Ltd.**

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Introduction

The low-affinity receptor for IgE (CD23) is involved in several aspects of T- and B-lymphocyte functions.[1] It is a transmembrane glycoprotein, at first reported to be selectively expressed on circulating B-cells with surface IgD and IgM,[2] but now known to be present on a variety of cell types: T lymphocytes, monocytes,[3] platelets,[4] and eosinophils.[5] Proteolytic cleavage of the extracellular domain of the CD23 antigen causes the release of several unstable soluble fragments and one stable soluble fragment (sCD23).[6] Soluble CD23 regulates IgE synthesis, promotes B- and T-cell growth, inhibits monocyte migration, and has recently been confirmed to have a co-stimulatory role in the activation of T-helper cells (Th).[7]

Recent studies suggest a functional division of human T-helper cells into Th1 and Th2. The Th2 subset is characterized by its ability to help B lymphocytes make antibodies. CD23, either in its soluble form or expressed on the surface of B lymphocytes, is a marker of activation of these cells. B-cell activation is likely to follow Th2 activity, and it has been proposed that increases in sCD23 or in the cell surface expression of CD23 by B lymphocytes may reflect Th2 activity. Although the mechanisms leading to upregulation of both CD23 cell-surface expression and sCD23 release are not completely understood, interleukin 4 (IL-4) has been reported to be a major inducer.[8–10] Diseases with enhanced T-cell-mediated immunity are associated with increased Th1 activity while those with augmented humoral immunity are characterized by increased Th2 activity. Augmented CD23 cell-surface expression and elevated serum sCD23 levels have been described in allergy, primary Sjogren's syndrome, systemic lupus erythematosus, rheumatoid arthritis, and autoimmune thyroiditis,[8–11] diseases associated with autoantibody production and B-cell hyperactivity.

Type 1 diabetes mellitus (DM) is caused by specific autoimmune destruction of pancreatic β-cells, involving both humoral and cellular immune mechanisms.[12] It is

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a disease that frequently involves a lengthy prodrome accompanied by pleomorphic immune interactions of autoreactive T cells and cytokines. The purpose of the present study was to assess serum sCD23 levels and CD23 expression on peripheral blood mononuclear cells (PBMC) and their regulation by T lymphokines such as interleukin 2 (IL-2) and IL-4 in subjects at increased risk of developing Type 1 DM and in newly diagnosed and long-standing Type 1 diabetic patients.

Subjects and Methods

Subjects

We measured serum sCD23 levels in 18 unaffected first-degree relatives (9 male and 9 female), aged 2.6–22 years and in 59 Type 1 diabetic patients (32 male and 27 female), aged 2.1–26 years. We considered all our 6 first-degree relatives with presence of islet cell antibodies (ICA) and 12 randomly selected relatives without ICA. At the time of the study, 24 patients were at clinical onset of Type 1 DM (<1 month from diagnosis of disease) and 35 had established or long-standing disease (median duration: 4.3 years, range: 2.1–13 years). The patients first tested at diagnosis were also tested after 2 years. Thirty-seven healthy subjects (17 male and 20 female), aged 3.1–28 years, served as controls. In all the first-degree relatives, 22 of the diabetic patients (12 newly diagnosed and 10 long-standing) and 20 healthy subjects, we also evaluated CD23 expression on PBMC and sCD23 spontaneous release in supernatants of PBMC activated with polyclonal activator (pokeweed mitogen -PWM), used either alone or in combination with the interleukins, IL-2 or IL-4. In all these subjects we also measured IL-4 in serum and culture supernatants.

All patients and subjects were tested for specific and total IgE. HbA_{1c} levels, HLA typing, islet cell antibodies (ICA), anti-gliadin (AGA) and other organ- and non-organ-specific antibodies were measured and insulin-autoantibodies (IAA) in relatives and in newly diagnosed patients, and insulin-antibodies (IA) in long-standing patients were also assayed. In all first-degree relatives an intravenous glucose tolerance test (IVGTT) was also performed.

All patients were receiving human insulin. Patients and subjects with familial history of allergic asthma, rhinitis and conjunctivitis were excluded. Informed consent was obtained from each subject and the study was approved by the Ethics Committee of our Hospital.

Detection of IL-4

IL-4 in sera and supernatants was quantified using an immunoenzymetric method (Medgenix diagnostics). Briefly, samples were allowed to react with capture antibodies coated on the microtitre plate and with monoclonal antibodies labelled with horseradish peroxidase. After incubation and washings, substrate solution were added and incubated for 30 min at room temperature. Absorbance was then read at 450 nm. IL-4 concentrations in samples were determined by interpolation from the standard curve. Results were expressed as pg ml^{-1}. The sensitivity of this method was estimated to be 0.2 pg ml^{-1}. The intra- and inter-coefficient of variation was 2.9 % and 5.2 %, respectively.

Detection of sCD23

Detection of sCD23 in sera and supernatants was carried out with an immunoenzymetric method (Medgenix diagnostics). Serial dilutions of the sCD23 standard and samples were allowed to react with capture antibodies coated to the microtitre plate and with horseradish-peroxidase conjugated monoclonal antibody. The antibodies used were specific for different epitopes of CD23. Results were expressed as U ml^{-1}. The sensitivity of this method was estimated to be 0.15 U ml^{-1}. The intra- and inter-coefficient of variation was 3.2 % and 4.6 %, respectively.

Cell Preparation and Culture

PBMC were isolated by Ficoll-Hypaque density gradient centrifugation and resuspended in RPMI 1640 medium (Gibco) supplemented with heat inactivated 10 % FCS, 2mM l-glutamine and 50 μg ml^{-1} gentamicin. 10^5 Cells per well were cultured in triplicate at a final volume of 200 μl. PBMC were stimulated with PWM (Gibco) at 1:200 dilution, alone or in combination with 20 units ml^{-1} human recombinant IL-2 or 250 units ml^{-1} human recombinant IL-4. Supernatants were harvested after 10 days of incubation at 37 °C in 5 % CO_2 air humidified environment.

Surface CD23 Analysis

CD23 cell surface expression was assessed using a phycoerythrin (PE)-conjugated monoclonal antibody specific for CD23 antigen (Becton Dickinson). The following fluoresceinated (FITC) monoclonal antibodies were used for phenotyping: anti-CD20, a marker of B cells, anti-CD14, a marker of monocytes and anti-CD2, a marker of T lymphocytes (Becton Dickinson). Phenotypic analysis of cell populations was performed by direct immunofluorescence in two-colour analysis using directly labelled antibodies on a FACScan flow cytometer (Becton Dickinson).

Results were expressed as the percentage of positive cells and as the mean fluorescence intensity (MFI) calculated as:

$$10^{(4\times \text{MFI reading})/1023}$$

Antibody Determination

ICA, anti-parietal cell antibodies and non-organ-specific (anti-nuclear, anti-mitochondrial, anti-reticulin, anti-

liver/kidney microsome, anti-smooth muscle) antibodies were assessed by indirect immunofluorescence as previously described.[13,14] ICA were considered positive when the titres were ≥ 10 Juvenile Diabetes Foundation (JDF) units. Anti-microsomal and anti-thyroglobulin antibodies were determined by passive haemoagglutination (Wellcome kits), IAA and IA by radioimmunoassay,[15] and anti-gliadin antibodies by ELISA.[16]

Metabolic Control, IVGTT and HLA Typing

HbA_{1c} levels were evaluated by HPLC (Biorad). Normal values were 5.14 ± 0.84 % (mean ± SD). IVGTT was performed according to Italian Society of Pediatric Endocrinology and Diabetology protocol.[17] Patients were HLA typed (class I and class II) by the microlymphocytotoxicity technique.[18]

Statistical Analysis

Results were compared by the non-parametric Wilcoxon's test. The relationship between sCD23 levels, percentage of CD23 cell surface expression and chronologic age, duration of Type 1 diabetes mellitus, presence of antibodies and HLA types was evaluated by Spearman rank correlation.

Results

Serum sCD23

Serum sCD23 levels were significantly higher in first-degree relatives (median: 3.2, range: 1.8–5.6 U ml^{-1}) ($p < 0.001$) and in newly diagnosed (median: 3.3, range: 0.4–5.5 U ml^{-1}) ($p < 0.001$) and long-standing (median: 2.5, range: 0.5–4.9 U ml^{-1}) ($p = 0.01$) Type 1 diabetic patients than in controls (median: 1.2, range: 0.9–4 U ml^{-1}) (Figure 1). No difference was observed between relatives and diabetic subjects. Newly diagnosed patients showed higher levels than long-standing patients ($p = 0.026$). No difference was observed in sCD23 levels after 2-year follow-up in patients evaluated at diagnosis. Serum sCD23 levels did not correlate with chronologic age or metabolic control (HbA_{1c}), but showed an inverse correlation with disease duration ($r = -0.39$, $p = 0.005$). No association was observed between serum sCD23 levels and HLA haplotypes in relatives and Type 1 diabetic patients.

ICA ≥ 10 JDF units were present in 6 (33 %) first-degree relatives and in 17 (71 %) newly diagnosed diabetic patients, and negative in all the long-standing patients. IAA were present in 2 (11 %) first-degree relatives and in 7 (29 %) newly diagnosed patients. IA were present in 28 (80 %) long-standing Type 1 diabetic patients. Overall positivity for at least one of the other organ-specific and non-organ-specific antibodies studied was 28 % in newly diagnosed and 32 % in long-standing patients. No differences were found in the sCD23 levels between relatives and patients with or without ICA or other antibodies. In terms of metabolic status in first-degree relatives, only one sibling showed an insulin response to IVGTT less than 1 ° centile. Three subjects have since developed diabetes.

Detection of sCD23 in Supernatants

No difference in sCD23 levels spontaneously produced by PBMC was observed in first-degree relatives and diabetic patients compared to controls (Table 1). Stimulation of PBMC by PWM and IL-2 alone or in combination did not enhance sCD23 secretion (data not shown). In contrast, stimulation with IL-4 alone induced a similar significant increase over baseline levels in all the groups examined (Table 1). Addition of PWM to PBMC inhibited the IL-4-induced sCD23 production (data not shown).

IL-4 in Sera and Supernatants

We evaluated IL-4 concentrations in serum and culture supernatants of 18 first-degree relatives, 22 Type 1 diabetic patients and 20 controls. Serum IL-4 levels were similar in all the groups considered (Figure 2). Levels of IL-4 spontaneously released in culture supernatants were significantly higher in newly diagnosed ($p = 0.022$) and long-standing ($p = 0.042$) diabetic patients than in controls, while no difference was observed between first-degree relatives and controls (Table 1). Stimulation of PBMC by PWM did not enhance IL-4 release (Table 1).

Cell Surface CD23

The percentages of CD23+ CD2+ and CD23+ CD14+ cells were similar in first-degree relatives, newly diagnosed and long-standing Type 1 diabetic patients and controls, while the percentage of B lymphocytes expressing CD23 was significantly higher in relatives (median 48.6, range 35–60 %) and in newly diagnosed (median 58, range 29.4–71 %) and long-standing (median 44.8, range 25–75.5 %) diabetic patients than in controls (median 28.5, range 20.8–42.2 %; $p < 0.001$, $p < 0.001$ and $p = 0.03$, respectively) (Figure 3). Newly diagnosed Type 1 diabetic patients showed also a greater CD23 cell surface expression evaluated as MFI (median 6.08, range 3.19–9.9) than first-degree relatives (median 3.4, range 2.75–6.25) ($p = 0.001$) and controls (median 3.8, range 3.2–6.3) ($p = 0.027$), while no difference was found between newly diagnosed and long-standing patients (median 4.45, range 2.9–8.3). No significant association was observed between CD23 cell surface expression and HLA haplotypes in relatives and IDDM patients. CD23 cell surface expression did not correlate with chronologic age or disease duration or metabolic control (HbA_{1c}). Soluble CD23 serum levels correlated with the percentage of CD23+ B cells ($r = 0.59$, $p = 0.004$).

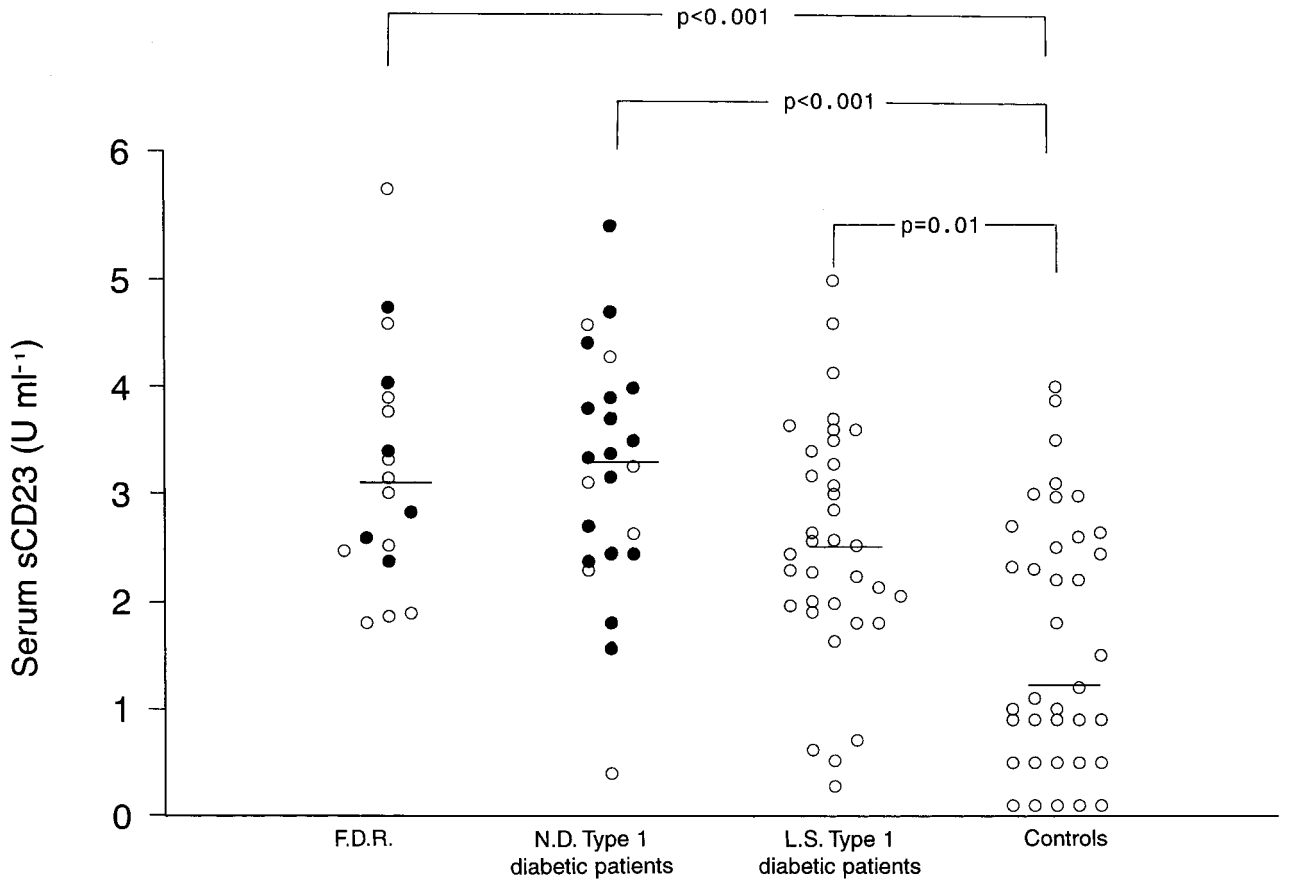

Figure 1. Serum sCD23 levels in first-degree relatives (F.D.R.), newly diagnosed (N.D.), and long-standing (L.S.) Type 1 diabetic patients and controls. • ICA ≥ 10 JDF units; ° ICA <10 JDF units. Horizontal bar represents median value

Table 1. Soluble CD23 and IL-4 levels in supernatants of cultured PBMC from first-degree relatives (F.D.R.), newly diagnosed (N.D.) and long-standing (L.S.) Type 1 diabetic patients and controls

	sCD23 (U ml^{-1})		IL-4 (pg ml^{-1})	
	Baseline	+IL-4	Baseline	+PWM
F.D.R.	0.65 (0–2.9)	6.38 (3.9–19.7)	7.4 (3.7–14)	6.8 (3.7–13.7)
N.D. Type 1 diabetic patients	1.73 (0–2.7)	8.26 (5.6–10.3)	20.15[a] (0–22.8)	17.8 (0–26.0)
L.S. Type 1 diabetic patients	2.6 (0.4–3.3)	7.8 (5.2–13.6)	21.15[b] (5.7–22.3)	19.6 (5.7–23.3)
Controls	1.64 (0–2.57)	6.18 (3.05–6.4)	6.8 (0–18.9)	0 (0–21.9)

Results are reported as median and range.[a] $p = 0.22$; [b] $p = 0.042$.

Discussion

Recent studies suggest a functional division of human T-helper cells into Th1 and Th2 based on their cytokine production profile: Th1 cells secrete IL-2 and IFN-γ, while Th2 cells secrete IL-4, IL-5, IL-6 and IL-10.[19–20] Evidence for an imbalance between Th1 and Th2 subsets in Type 1 diabetes mellitus is provided by the finding of autoantibodies and cellular immune responses to β-cell antigens.[21] Direct evidence for involvement of the Th2 subset in NOD mice has been reported.[22] It has been suggested that serum levels of sCD23 could provide an indirect means of assessing the overall balance of Th1 and Th2 activity.[10] The elevated sCD23 levels and the increased percentage of cells expressing CD23 observed in our subjects at increased risk of Type 1 diabetes mellitus and in newly diagnosed diabetic patients compared to controls are compatible with the presence

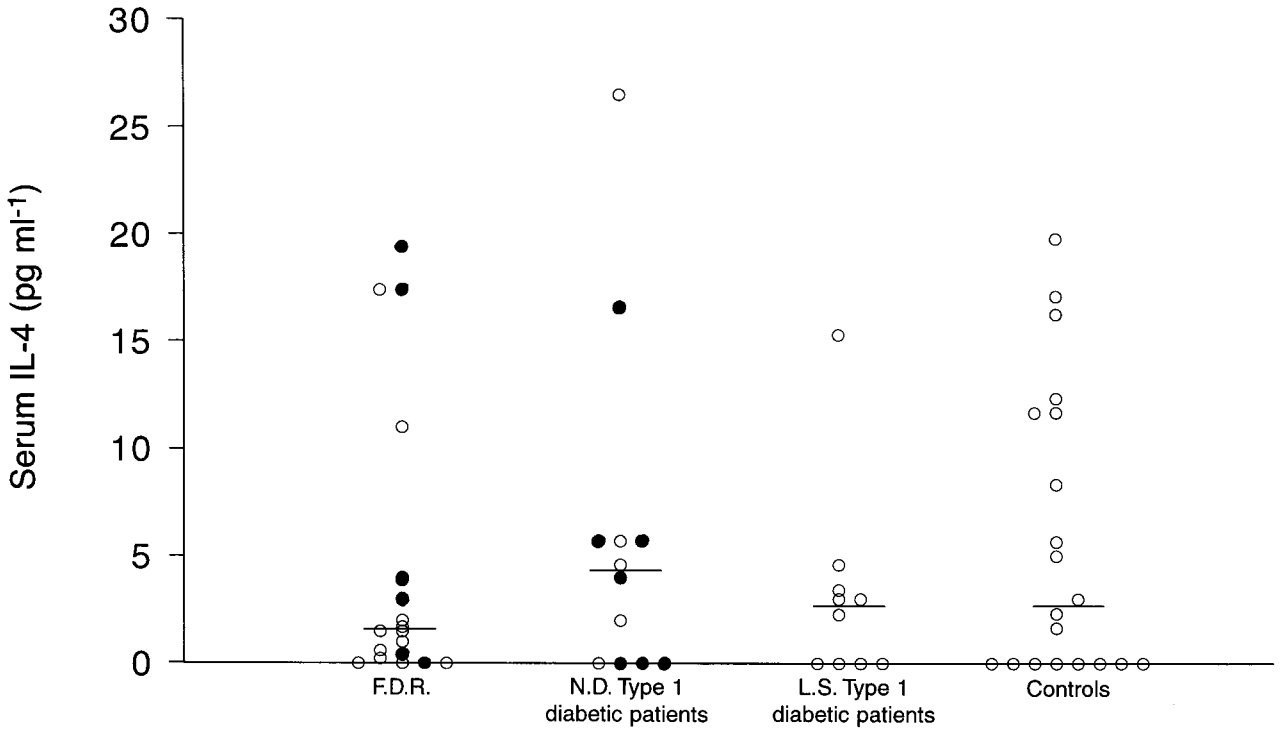

Figure 2. Serum IL-4 levels in first-degree relatives (F.D.R.), newly diagnosed (N.D.), and long-standing (L.S.) Type 1 diabetic patients and controls. • ICA ≥ 10 JDF units; ◦ ICA <10 JDF units. Horizontal bar represents median value

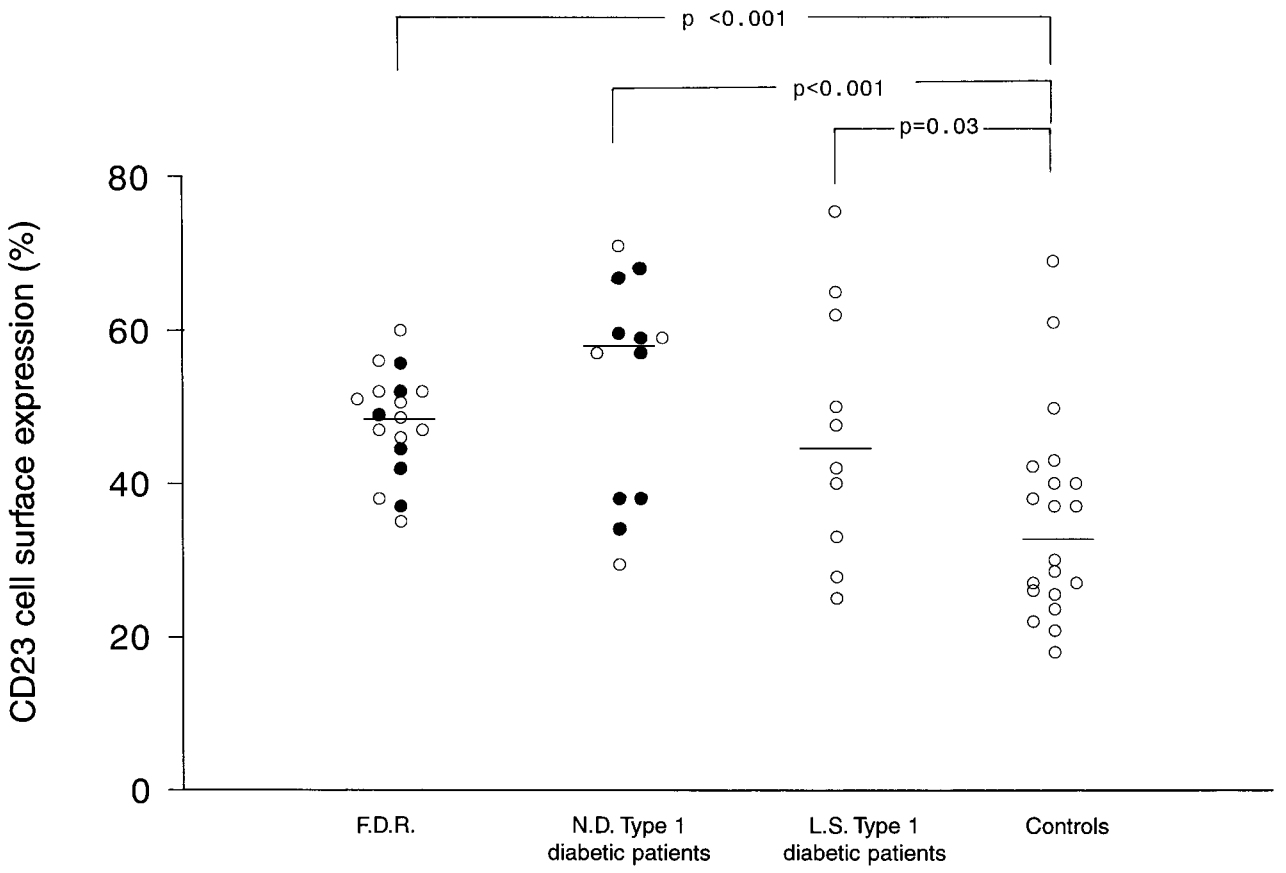

Figure 3. Percentage of CD23 cell surface expression in first-degree relatives (F.D.R.), newly diagnosed (N.D.), and long-standing (L.S.) Type 1 diabetic patients and controls. • ICA ≥ 10 JDF units; ◦ ICA <10 JDF units. Horizontal bar represents median value

of a hyperactive immune system reported in prediabetic and newly diagnosed Type 1 diabetic patients.[23–25] Such a state has been previously invoked as a possible predisposing factor to autoimmune disease.[26] The significant enhancement of both sCD23 levels and CD23 expression in newly diagnosed compared to long-standing diabetic subjects reflects the transient humoral immune alterations observed at the onset of disease.[12,27,28] However, this state persists for some years; in fact, sCD23 levels evaluated at onset of disease did not significantly decrease after 2 years. Increased sCD23 levels and percentage of cells expressing CD23 found in our subjects at risk of diabetes (as well as other autoantibodies), in a preclinical phase, months before the onset of diabetes, suggests that Type 1 diabetes results from a chronic autoimmune process.[12] Serum sCD23 levels were similar at 6 months before, at onset, and 2 years later. However serum sCD23 values were increased in the serum sample at 1 month before the clinically overt disease, available in only one patient (data not shown). The observation of higher IL-4 levels spontaneously released in supernatants of cultured PBMC from newly diagnosed and long-standing Type 1 diabetic patients than from controls, together with the increased levels of sCD23 and the augmented percentage of cell expressing CD23 molecule, suggest a probable involvement of Th2 subset in the pathogenesis of Type 1 diabetes mellitus. However, there is only indirect evidence that CD23 levels reflect an imbalance of Th1/Th2 activity, and whether our observations are directly related to disease pathogenesis, or to secondary immune responses to antigens released from damaged islets, remained to be determined.

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